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**PALM INTRANET**

## Continuity Information for 10/068612

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### Child Data

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Contents

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Search Another: Application#

Search

or Patent#  Search

PCT /  /  Search

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Search

Attorney Docket #  Search

Bar Code #  Search

To go back use Back button on your browser toolbar.

Back to [PALM](#) | [ASSIGNMENT](#) | [OASIS](#) | [Home page](#)

selected samples of H1 colonies in the well were scraped off with a blunt-ended Pasteur pipette under a dissecting microscope. The cell colony suspension was spun briefly to precipitate the cells followed by removal of the supernatant. 1% NP-40 100  $\mu$ l was added to the cell pellet, which was followed by vortexing until total lysis was obtained. 850  $\mu$ l LB medium and 50  $\mu$ l Protease Inhibitor Cocktail (Sigma) were added to the lysate to achieve a total volume of 1 ml. An aliquot of the lysate was used to make serial dilutions for plaque assay in LB agar-coated plates (some of the plates with 100-300 plaques were used for plaque lift as needed). Whenever necessary, the remaining lysate was amplified and then saved for the next round of panning according to the manufacturer's instruction (Novagen). A total of 4 rounds of panning were carried out for MEF phage panning, and this was followed by sequencing analysis of the selected phage.

**[0030] Sequencing of phage plaque.** The phage lysate from the fourth round of panning with the MEF phage library was plated at a dilution sufficient to produce about 200 plaques per plate. Plaque pick-up, PCR amplification, and sequencing were performed according to the manufacturer's instruction (Novagen). Briefly, more than 100 plaques were randomly picked up by using a pipette tip to scrape up a portion of the top of an individual plaque and to dispense the plaque in a tube containing 100  $\mu$ l of 10 mM EDTA (pH 8.0). The tube was vortexed briefly, heated to 65°C for 10 min., cooled to RT, and centrifuged at 14,000 g for 3 min. to clarify. Inserts in the phage lysate were amplified with a pair of primers complementary to the flanking regions of the T7Select 10-3 Arms. The PCR reaction products were electrophoresized on TAE gel. Only for those PCR reactions for which the products exhibited clear single insert bands (about 200 reactions per phage lysate) were the corresponding plaques chosen for sequencing. Single chain DNAs and nucleotides left in the reactions were digested with a Pre-sequencing kit (USB, Cleveland, OH) followed by heat inactivation of the enzymes. An aliquot from each of the treated PCR reactions was then sequenced with a forward primer (TGC CAA TAA AGG TGA GGG TA). The sequences were visualized by the EditView software and analyzed by BLAST in the GenBank.

**[0031] PCR detection of gene inserts in phage.** PCR amplification of MEF cDNA inserts in the phage was performed mainly according to the protocol (Novagen). Briefly, phage DNA was purified from  $2 \times 10^{10}$  pfu of phage using a PCR purification kit (Qiagen) and an